# SYNTHESIS, CHARACTERIZATION, IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF CHALCONE DERIVATIVES OF 4-HYDROXYACETOPHENONE

**BY**

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## CERTIFICATION

This is to certify that this research was carried out by Kelani,MonsuruTemitope in the Department of Chemistry, Faculty of Science, ObafemiAwolowo University, Ile-Ife, under the supervision of Professor C. A. Obafemi.

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Supervisor Date

(Professor C. A. Obafemi)

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Professor A. O. Ogunfowokan Date

(Head of Department)

## DEDICATION

This thesis is dedicated to my late father, AlhajiKelaniAkanbi.

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Scheme

## ABBREVIATIONS

| | |
|---|---|
| AcOH: | Acetic acid |
| EtOH: | Ethanol |
| $D_2O$: | Deuterium oxide/heavy water |
| DMSO: | Dimethyl sulphoxide |
| STD: | Standard |
| $\lambda_{max}$: | Maximum wavelength |
| OD: | Optical density |
| NCIB: | National Collection of Industrial Bacteria |
| LIO: | Locally Induced Organism |
| STREP: | Streptomycin |
| AMP: | Ampicillin |

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## ABSTRACT

This study synthesizedsome phenolic chalcone-ninhydrin adducts via microwave-irradiation of phenolic chalcones with ninhydrin. This was with a view to improving the chemotherapeutic property of chalcones.

The chalcones were prepared by reacting 4-hydroxyacetophenone with an equivalent mole of sevensubstituted benzaldehydesto afford the corresponding chalcones (**1a**, **2a**, **3a**, **4a**, **5a**, **6a** and **7a**). The resulting phenolic-chalcones were reacted with ninhydrin by pulse microwave-irradiationof the reactants in glacial acetic acid for 8 min to give the following compounds **1b**, **2b**, **3b**, and **4b**, except the reaction of **7a** with ninhydrin which was achieved by refluxing in glacial acetic acid for 6 hours to yieldninhydrin adduct of the chacone**7b**.The compounds were screened for antioxidant activities which included2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) and (ferric reducing antioxidant power)FRAP assays. They were also screened *in-vitro* against nine strains of Gram-positive and five Gram-negative bacteria, to determine their zones of inhibition, minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC).

The structures of some of the synthesized compounds were investigated and confirmed using nuclear magnetic resonance (NMR) experiments ($^{1}H$, $^{13}C$, and distortionless enhancement by polarization transfer (DEPT). Some of the compounds were also subjected to infra-red spectroscopic analysis and mass spectrometry to corroborate the information obtained from the NMR experiments. The spectroscopic analysis showed that the expected structures of the chalcones were obtained and that the ninhydrin adducts were either di- or tri-substituted adducts.Compound **7a** demonstrated the best DPPH radical scavenging ability with an $IC_{50}$ value

of 0.0240 mg/ml which was lower than the standard (ascorbic acid, 0.0250 mg/ml).In the FRAP assay, however, compound **4b** demonstrated the best activity with an ascorbic acid equivalent of (AAE) of 0.470 mg. Therefore,compounds **7a** and **7b** exhibited a comparable radical scavenging ability to the ascorbic acid used as standard drug than the other compounds against the DPPH, while compound **4b** exhibited an excellent ferric reducing antioxidant ability using the FRAP assays. All the bacterial strains were sensitive to compound **5a** except *Pseudomonasaeruginosa,*which was also not susceptible to the standard drugs, streptomycin and ampicillin. Compound **5a** exhibited the best activity against the Gram-negative bacteria and also had the lowest MIC values than ampicillin.

The study concluded that compounds **4a**, **7a** and **7b** could serve as good antioxidant agents and compound **5a**exhibited an excellent antimicrobial property.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background to the Study

Chalcones are also known as benzalacetophenone or benzylidene acetophenone. In these compounds, two aromatic rings are linked by an aliphatic three carbon chain.Structurally, they are a group of compounds bearing 1,3-diaryl-2-propen-1-one skeletal framework, which consists of open-chain flavonoids in which the two aromatic rings are joined together by three carbons in an$\alpha,\beta$-unsaturated system. Chemically, they can be easily cyclized by a Michael addition at the$\beta$position of the carbonyl to form a flavanone. The presence of double bond results in *cis* and*trans* isomers of chalcones in nature, of which thetransform is thermodynamically stable. Chalconesare one of the most structurally diverse groups of flavonoids existing as dimers, oligomers, Diels-Alder adducts, and conjugates of various kinds. Additionally, the attachment of varieties of hydroxyl, methoxy, and alkenyl functionalities to the framework of chalcone accounts for its structural diversity as well (Andersen *et al*., 2006).Chalcones derivatives are an important class of compounds possessing diverse biological properties including antiplasmodial (Frolich *et al*., 2005), antibacterial (Parekh *et al*., 2005), trypanocidal and leishmanicidal properties (Lunarcdi *et al*., 2003), antitumour (Tatematsu, Min *et al*., 1996), antiinflammatory (Williams *et al*., 1995), antiviral activites (Mahmood *et al*., 1997). Extensive work on synthesis of chalcones has been done by variousmethods. They are precursors of flavones in the biosynthesis of flavonoids.

### 1.1.2 BIOSYNTHESIS OF CHALCONES

The most common sequence uses three malonyl CoA acylations followed by cyclization to a new
aromatic ring. The simplest type is exemplified by resveratrole, the compound in red wine that helps
to prevent heart disease (fig. 1.0, Schroder, 1999).

A different cyclization leads to the flavones and anthocyanidins. Reaction of the stable enol from a

1,3-diketone with the thiol ester as electrophile results in acylation at carbon in the manner of the Claisen ester condensation with loss of CoASH and the formation of a trihydroxybenzene ring. The biosynthesis of 2',4',6',4-tetrahydroxychalcone, also known as chalconaringenin will be the focus here. This compound is formed by the condensation of three molecules of malonyl coenzyme A (malonyl coA) with one mole of p-coumaroyl-CoA catalyzed by chalcone synthase (Schroder, 1999) which gives a tetraketide precursor. Subsequent cyclization of this tetraketide precursor yields the chalcone. Natural chalcones represent the convergence of two biosynthetic pathways, the acetate (leading to the ring A) and the shikimate (leading to ring B), respectively. Studies indicate that the extent of B-ring hydroxyl substitution in flavonoids is controlled at the $C_{15}$ level rather than incorporation of specific cinnamoyl-CoA derivatives (Forkmann and Heller, 1999). The formation of B ring results from p-coumaroyl-CoA which is achieved through hydroxylase and methyl transferase enzymes and the existence of a specific 3-hydroxylase for chalcones is from the general flavonoids 3'-hydroxylase (Wimmer *et al.,* 1998) which are cytochrome P450-dependent monooxygenases. The number of hydroxyl groups in the ring A is also controlled, for example, in isoliquiritigenine (2',4',4-trihydroxychalcone) found in *leguminosae* is most likely to be formed via a reduction step at the polyketide level, catalyzed by a NADPH-dependent monomeric enzyme known as chalcone ketide or polyketide reductase (Schroder, 1999).

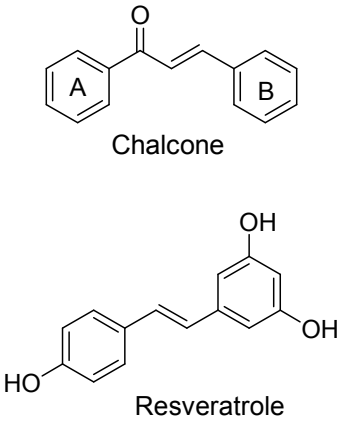


**Fig. 1.0** The structure of a typical chalcone and resveratrole